(Document name)

Specification

(Title of the invention)

NOVEL DIFFERENTIATION INDUCING PROCESS OF EMBRYONIC STEM CELL TO ECTODERMAL CELL AND ITS USE

(Scope of the claims)

(Claim 1) A method for inducing differentiation of an embryonic stem cell into an ectodermal cell, which comprises culturing the embryonic stem cell under non-aggregation conditions.

(Claim 2) The method according to claim 1, wherein the ectodermal cell is a cell capable of differentiating into a nervous system cell or an epidermal system cell.

(Claim 3) A method for inducing differentiation of an embryonic stem cell into an ectoderm-derived cell, which comprises culturing the embryonic stem cell under non-aggregation conditions.

(Claim 4) The method according to claim 3, wherein the ectoderm-derived cell is a nervous system cell or an epidermal system cell.

(Claim 5) The method according to claim 4, wherein the epidermal system cell is an epidermal cell.

(Claim 6) The method according to any one of claims 1 to 5, wherein said culturing is carried out in the presence of bone morphogenetic protein 4.

(Claim 7) The method according to claim 4, wherein the nervous system cell is a neural stem cell or a nerve cell.

(Claim 8) The method according to claim 7, wherein the nerve cell is a nerve cell selected from the group consisting of the following (a), (b), (c) and (d):

(a) a dopaminergic neuron;

(b) an acetylcholinergic neuron;

(c) a γ-aminobutyratergic neuron; and

(d) a serotonergic neuron.

(Claim 9) The method according to any one of claims 1 to 8, wherein the non-aggregation conditions are conditions not mediating an embryoid body.

(Claim 10) The method according to any one of claims 1 to 9, which further comprises culturing under serum-free culture conditions.

(Claim 11) The method according to any one of claims 1 to 10, wherein said culturing is carried out in the presence of a stroma cell-derived factor.

(Claim 12) The method according to any one of claims 1 to 11, wherein said culturing is carried out in the presence of a stroma cell.

(Claim 13) The method according to claim 12, wherein the stroma cell is a stroma cell whose proliferation potency is deleted by a physicochemical treatment.

(Claim 14) The method according to claim 13, wherein the physicochemical treatment is selected from the group consisting of the following (a), (b) and (c):

(a) a treatment with an antitumor agent;

(b) a treatment by an radiation irradiation; and

(c) a treatment for tissue fixation used in pathologic diagnosis.

(Claim 15) The method according to claim 14, wherein the antitumor agent is selected from the group consisting of mitomycin C, 5-fluorouracil, adriamycin and methotrexate.

(Claim 16) The method according to claim 14, wherein the treatment for tissue fixation used in pathologic diagnosis is selected from the group consisting of a microwave fixation, a rapid freeze-substitution fixation, a glutaraldehyde fixation, a p-formaldehyde fixation, a formalin fixation, an acetone fixation, a Van fixation, a periodic acid fixation, a methanol fixation and an osmic acid fixation.

(Claim 17) The method according to any one of claims 11 to 16, wherein the stroma cell is selected from the group consisting of the following (a), (b), (c), (d), (e), (f) and (g):

(a) a fetal primary culture fibroblast;

(b) an SIHM mouse-derived STO cell;

(c) a mouse fetus-derived NIH/3T3 cell;

(d) an M-CSF deficient mouse calvaria-derived OP9 cell;

(e) a mouse calvaria-derived MC3T3-G2/PA6 cell;

(f) an embryonic stem cell-derived stroma cell; and

(g) a bone marrow mesenchymal stem cell-derived stroma cell.

(Claim 18) The method according to any one of claims 1 to 17, wherein the embryonic stem cell is selected from the group consisting of the following (a), (b) and (c):

(a) an embryonic stem cell established by culturing an early embryo before implantation;

(b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and

(c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using a gene engineering technique.

(Claim 19) The method according to any one of claims 1 to 18, wherein said culturing is carried out in the absence of retinoic acid.

(Claim 20) The method according to any one of claims 1 to 19, wherein the embryonic stem cell is differentiated into an ectodermal cell or an ectoderm-derived cell at an efficiency of 5% or more.

(Claim 21) The method according to any one of claims 1 to 20, which does not substantially accompany differentiation induction of a mesodermal system cell.

(Claim 22) A medium for inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which is used in the method according to any one of claims 1 to 21.

(Claim 23) An agent for inducing differentiation of an ectodermal cell into an epidermal system cell, which comprises, as an active ingredient, bone morphogenetic protein 4.

(Claim 24) An agent for inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises, as an active ingredient, a stroma cell which has activity of inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, or a factor derived from the cell.

(Claim 25) The agent for inducing differentiation according to claim 24, wherein the stroma cell is the stroma cell described in claim 24.

(Claim 26) The agent for inducing differentiation according to claim 24 or 25, wherein the stroma cell-derived factor is capable of adsorbing a mucopolysaccharide.

(Claim 27) The agent for inducing differentiation according to claim 26, wherein the mucopolysaccharide is heparin.

(Claim 28) A medium supernatant obtained by culturing a stroma cell which has activity of inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell in a medium comprising a mucopolysaccharide.

(Claim 29) The medium supernatant according to claim 28, wherein the stroma cell is the stroma cell described in any one of claims 13 to 17.

(Claim 30) The medium supernatant according to claim 28 or 29, wherein the mucopolysaccharide is heparin.

(Claim 31) An agent for inducing differentiation of an ectodermal cell or an ectoderm-derived cell, which comprises, as an active ingredient, the culture supernatant described in any one of claims 28 to 30.

(Claim 32) A method for obtaining an antibody which specifically recognizes a stroma cell which has activity of inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises using a stroma cell as an antigen.

(Claim 33) The method according to claim 32, wherein the stroma cell is a stroma cell described in any one of claims 13 to 17.

(Claim 34) An antibody which specifically recognizes a stroma cell which has activity of inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which is obtained by the method according to claim 32 or 33.

(Claim 35) A method for obtaining an antigen recognized by the antibody according to claim 34, which comprises using the antibody.

(Claim 36) An antigen recognized by the antibody according to claim 34, which is obtained by the method according to claim 35.

(Claim 37) A medium for culturing a cell, which comprises the antigen according to claim 36.

(Claim 38) An ectodermal cell or an ectoderm-derived cell, which is induced by using the method according to any one of claims 1 to 21.

(Claim 39) A method for increasing purity of a cell which is differentiation-induced from an embryonic stem cell, which comprises culturing the ectodermal cell or ectoderm-derived cell according to claim 38 in a medium comprising an antitumor agent.

(Claim 40) The method according to claim 39, wherein the antitumor agent is selected from the group consisting of mitomycin C, 5-fluorouracil, adriamycin, methotrexate and ara-C.

(Claim 41) A cell which is obtained by using the method according to claim 39 or 40.

(Claim 42) A method for evaluating a substance relating to the regulation in a differentiation step from an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises: carrying out the method according to any one of claims 1 to 21 in the presence of a substance to be tested and the method in the absence of the substance to be tested; and comparing the differentiation step from an

embryonic stem cell into an ectodermal cell or an ectoderm-derived cell in the presence of the substance to be tested with that in the absence of the substance to be tested.

(Claim 43) A method for screening a substance relating to the regulation in a differentiation step from an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises: carrying out the method according to any one of claims 1 to 21 in the presence of a substance to be tested and the method in the absence of the substance to be tested; and comparing the differentiation step from an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell in the presence of a substance to be tested with that in the absence of the substance to be tested.

(Claim 44) A method for evaluating a substance relating to the regulation of the function of an ectodermal cell or an ectoderm-derived cell, which comprises: culturing the cell according to claim 38 in the presence of a substance to be tested and the cell in the absence of the substance to be tested; and comparing the function of an ectodermal cell or an ectoderm-derived cell in the presence of the substance to be tested with that in the absence of the substance to be tested.

(Claim 45) A method for screening a substance relating to the regulation of the function of an ectodermal cell or an ectoderm-derived cell, which comprises: culturing the cell according to claim 38 in the presence of a substance to be tested and that in the absence of the substance to be tested; and comparing the function of the ectodermal cell or the ectoderm-derived cell in the presence of the substance to be tested with that in the absence of the substance to be tested.

(Claim 46) A medicament comprising a stroma cell having activity of inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, or a factor derived from the cell.

(Claim 47) The medicament according to claim 46, wherein the stroma cell is a stroma cell described in any one of claims 13 to 17.

(Claim 48) A medicament comprising the antibody according to claim 34.

(Claim 49) A medicament comprising the antigen according to claim 36.

(Claim 50) A medicament comprising the ectodermal cell or an ectoderm-derived cell according to claim 38.

(Claim 51) A medicament comprising the cell according to claim 41.

(Claim 52) The medicament according to any one of claims 46 to 51, which is a medicament for diagnosing, preventing and/or treating diseases caused by the ectoderm-derived cell.

(Claim 53) The medicament according to claim 52, wherein the diseases caused by the disorder of an ectoderm-derived cell are diseases caused by the disorder of a nervous system cell or an epidermal system cell.

(Claim 54) The medicament according to claim 53, wherein the diseases caused by the disorder of a nervous system cell are Alzheimer disease, Huntington chorea, Parkinson disease, ischemic cerebral disease, epilepsy, brain injury, vertebral injury, motor neuron disease, neurodegeneration disease, pigmentary retinal dystrophy, cochlear hearing loss, multiple sclerosis, amyotrophic lateral sclerosis or diseases due to a neurotoxin damage; and the diseases caused by the disorder of an epidermal system cell are burn, wound, healing of wound, compression gangrene or psoriasis.

(Claim 55) A method for immunologically detecting the antigen according to claim 46, which comprises using the antibody according to claim 34.

(Claim 56) A tissue immunostaining method of the antigen according to claim 46, which comprises using the antibody according to claim 34.

(Claim 57) A method for obtaining a stroma cell-derived factor which has activity of inducing differentiation of an embryonic stem cell into an ectoderm-derived cell, which comprises using, as an index, the activity of inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell.

(Claim 58) The method according to claim 57, which comprises: binding the stroma cell-derived factor to a mucopolysaccharide; and recovering the element from the stroma cell-derived factor bound to the mucopolysaccharide.

(Claim 59) The method according to claim 57 or 58, wherein the stroma cell is the stroma cell according to any one of claims 13 to 17.

(Claim 60) The method according to claim 58, wherein the mucopolysaccharide is heparin.

(Detailed description of the invention)

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(Technical field to which the invention belongs)

The present invention relates to a method for inducing differentiation of an embryonic stem cell into a functional cell. More particularly, the present invention relates to a process for inducing differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell useful for cell medical treatment, the differentiation-induced cell and use thereof. Also, the present invention relates to a medium used in the above process, an antibody which specifically recognizes a stroma cell in the above process, an antigen recognized by the antibody and use thereof.